REMARKS

Claims 86-115 are pending in this application. Applicants have amended claims 86-90 without prejudice or disclaimer. Claims 92-115 are new. Support for the claim amendments and new claims can be found in the specification as filed, for example, at page 4, lines 31-32; page 8, lines 19-24; page 8, line 33- page 9, line 2; page 12, lines 8-9; page 23, lines 6-7 and 28-30; page 29, lines 14-17; page 45, lines 27-30; page 65, lines 27-34; page 82, line 33- page 83, line 5; and page 86, lines 23-28. No new matter has been added.

Applicants submit that amended and new claims read on the Group X of the Restriction Requirement mailed May 31, 2006 that was elected for prosecution.

Election/Restriction

The Office at page 2 of the Office Action states that claim 91 most closely corresponds to non-elected invention Group VIII of the Restriction Requirement mailed May 31, 2006 and that claim 91 is therefore withdrawn as being drawn to a non-elected invention.

Applicants respectfully disagree. Group VIII of the Restriction Requirement was drawn to a pharmaceutical composition. In contrast, claim 91 recites a product made by the cell of the elected invention group (Group X: a sulfatase-producing cell). Because claim 91 depends from and thereby incorporates all of the elements of the examined claims, Applicants request that upon allowance of the examined claims, claim 91 be rejoined with the examined claims.

Specification

The Office at page 3 objects to the title of the application. In the interest of expediting prosecution, Applicants have amended the title as indicated herein. Withdrawal of the objection to the title is respectfully requested.

35 U.S.C. § 112, Second Paragraph

The Office at pages 3-4 alleges that claims 86-90 are indefinite because claim 86 recites the terms "a sulfatase with an increased expression" and "Formylglycine Generating Enzyme with an increased expression" without defining to what the increased level of expression is being compared.

As indicated herein, Applicants have amended claim 86 and submit that the amendments to the claim overcome the Office's rejection. Applicants respectfully request that this rejection of claim 86 (and its dependencies, claims 87-90) be withdrawn.

35 U.S.C. § 101

At page 4 of the Office Action, the Office rejects claims 86-90 for allegedly being directed to non-statutory subject matter and for reading on a product of nature.

As provided herein, claim 86 has been amended to recite, in part, that the cell expresses "a Formylglycine Generating Enzyme, wherein the Formylglycine Generating Enzyme is an activated form of an endogenous Formylglycine Generating Enzyme of SEQ ID NO:2 or an ortholog thereof or an exogenous Formylglycine Generating Enzyme of SEQ ID NO:2 or an ortholog thereof ..." Because a cell that expresses a Formylglycine Generating Enzyme that is an activated form of endogenous Formylglycine Generating Enzyme or an exogenous Formylglycine Generating Enzyme is not naturally occurring, the claims do not read on a product of nature. Applicants submit that amended claim 86 (and dependent claims 87-90) does not read on a product of nature and request that this rejection be withdrawn.

35 U.S.C. § 112, First Paragraph, Written Description

The Office at pages 5-8 alleges that claims 86-90 fail to satisfy the written description requirement. Specifically, the Office alleges that Applicants have failed to demonstrate possession of the Formylglycine Generating Enzyme that is expressed by the claimed cells.

In the interest of expediting prosecution, Applicants have amended claim 86 to recite that the cells express a Formylglycine Generating Enzyme of SEQ ID NO:2 or an ortholog thereof. Applicants submit that the specification demonstrates that Applicants had possession of this claimed enzyme.

As described in the specification, Applicants identified a gene that encodes Formylglycine Generating Enzyme (FGE), an enzyme responsible for the unique post-translational modification occurring on sulfatases that is essential for sulfatase function (formation of L-C_α-formylglycine; a.k.a. *FGly* and/or *2-amino-3-oxopropanoic acid*). Applicants discovered that mutations in the FGE gene lead to the development of Multiple Sulfatase Deficiency (MSD) in subjects. FGE enhances the activity of sulfatases such as

Iduronate 2-Sulfatase, Sulfamidase, N-Acetylgalactosamine 6-Sulfatase, N-Acetylglucosamine 6-Sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase D, Arylsulfatase E, Arylsulfatase F, Arylsulfatase G, HSulf-1, HSulf-2, HSulf-3, HSulf-4, HSulf-5, and HSulf-6 (see, e.g., page 3 of the specification).

Applicants have identified the human Formylglycine Generating Enzyme (SEQ ID NO:2) and have also identified numerous orthologs from other species. The specification sets out in detail the identifying characteristics of Formylglycine Generating Enzyme homologs such as orthologs (and how to distinguish orthologs from paralogs) such that a skilled practitioner would recognize that Applicants were indeed in possession of the claimed Formylglycine Generating Enzyme orthologs.

For example, Example 2 (pages 80-88) describes how both full length and EST fragment orthologs of SEQ ID NO:2, from both eukaryotes and prokaryotes, were identified. The example also describes how including highly conserved subdomain regions were identified. In part, Example 2 sets out:

> Signal peptides and cleavage sites were described with the method of von Heijne (*Nucleic Acids Res.*, 1986, 14:4683) implemented in EMBOSS (Rice et al., *Trends in Genetics*, 2000, 16:276-277), and the method of Nielsen et al. (*Protein Engineering*, 1997, 10:1-6). N-glycosylation sites were predicted using the algorithm of Brunak (Gupta and Brunak, *Pac. Symp. Biocomput.*, 2002, 310-22).
>
> Functional domains were detected by searching PFAM-Hidden-Markov-Models (version 7.8) (Sonnhammer et al., *Nucleic Acids Res.*, 1998, 26:320-322). Sequences from the PFAM DUF323 seed were obtained from TrEMBL (Bairoch, A. and Apweiler, R., *Nucleic Acids Res.*, 2000, 28:45-48). Multiple alignments and phylogenetic tree constructions were performed with Clustal W (Thompson, J., et al., *Nucleic Acids Res.*, 1994, 22:4673-4680). For phylogenetic tree computation, gap positions were excluded and multiple substitutions were corrected for. Tree bootstraping was performed to obtain significant results. Trees were visualised using Njplot (Perriere, G. and Gouy, M., *Biochimie*, 1996, 78:364-369). Alignments were plotted using the pret- typlot command from EMBOSS.
>
> To search for FGE homologs, the databases NR, NT and EST of the National Center for Biotechnology Information (NCBI) (Wheeler et al., *Nucleic Acids Res.*, 2002, 20:13-16), were queried with BLAST (Altschul et al., *Nucleic Acids Res.*, 1997, 25:3389-3402). For protein sequences, the search was performed using iterative converging Psi-Blast against the current version of the NR database using an expectation value cutoff of 10^{-40}, and default parameters. Convergence was reached after 5 iterations. For nucleotide sequences, the search was performed with Psi-TBlastn: using NR and the protein sequence of human FGE as input, a score matrix for hFGE was built with iterative converging Psi-Blast. This matrix was used as input for blastall to query the nucleotide databses NT and EST. For both steps, an expectation value cutoff of 10^{-20} was used.
>
> Protein secondary structure prediction was done using Psipred (Jones, D., *J Mol Biol.*, 1999, 292:1950-202; McGuffin, L., et al., *Bioinformatics*, 2000, 16:404-405).
>
> Similarity scores of the subdomains were computed from alignments using the cons algorithm form EMBOSS with default parameters. The metaalignments were generated by aligning consensus sequences of the FGE-family subgroups. Genomic loci

organisation and synteny were determined using the NCBI's human and mouse genome resources at NCBI (Bethesda, MD) and Softberry's (Mount Kisco, NY) Human- Mouse-Rat Synteny. Bacterial genome sequences were downloaded from the NCBI-FTP-server. The NCBI microbial genome annotation was used to obtain an overview of the genomic loci of bacterial FGE genes.

Results and Discussion

Basic features and motifs of human FGE and related proteins

The human FGE gene (SEQ ID NOs:1, 3) encodes the FGE protein (SEQ ID NO:2) which is predicted to have 374 residues. A cleavage signal between residues 22-33 (Heijne-Score of 15.29) and a hydropathy-score (Kyte, J. and Doolittle, R., *J Mol Biol.*, 1982, 157:105-132) of residues 17-29 between 1.7 and 3.3 indicate that the 33 N-terminal residues are cleaved off after ER-translocation. However with the algorithm of Nielsen et al. (*Protein Engineering*, 1997, 10:1-6), cleavage of the signal sequence is predicted after residue 34. The protein has a single potential N-glycosylation site at Asn 141.

A search with the FGE protein sequence against the protein motif database PFAM (Sonnhammer et al., *Nucleic Acids Res.*, 1998, 26:320-322) revealed that residues 87-367 of human FGE can be classified as the protein domain DUF323 ("domain of unknown function", PF03781) with a highly significant expectation value of $7{:}9{*}10^{-114}$. The PFAM-seed defining DUF323 consists of 25 protein sequences, of which the majority are hypothetical proteins derived from sequencing data. To analyse the relationship between human FGE and DUF323, a multiple alignment of FGE with the sequences of the DUF323 seed was performed. Based on this, a phylogenetic tree was constructed and bootstraped. Four of the hypothetical sequences (TrEMBL-IDs Q9CK12, Q9I761, O94632 and Q9Y405) had such a strong divergence from the other members of the seed that they prevented successfull bootstraping and had to be removed from the set. Figure 2 shows the bootstraped tree displaying the relationship between human FGE and the remaining 21 DUF323 seed proteins. The tree can be used to subdivide the seed members into two categories: homologs closely related to human FGE and the remaining, less related genes.

The topmost 7 proteins have a phylogenetic distance between 0.41 and 0.73 to human FGE. They only contain a single domain, DUF323. The homology within this group extends over the whole amino acid sequence, the greater part of which consists of the DUF323 domain. The DUF323 domain is strongly conserved within this group of homologs, while the other 15 proteins of the seed are less related to human FGE (phylogenetic distance between 1.14 and 1.93). Their DUF323 domain diverges considerably from the highly conserved DUF323-domain of the first group (cf. section "Subdomains of FGE and mutations in the FGE gene"). Most of these 15 proteins are hypothetical, six of them have been further investigated. One of them, a serine/threonine kinase (TrEMBL:O84147) from C. trachomatis contains other domains in addition to DUF323: an ATP-binding domain and a kinase domain. The sequences from R. sphaeroides (TrEMBL: Q9ALV8) and Pseudomonas sp.(TrEMBL: O52577) encode the protein NirV, a gene cotranscribed with the copper-containing nitrite reductase nirK (Jain, R. and Shapleigh, J., *Microbiology*, 2001, 147:2505-2515). CarC (TrEMBL: Q9XB56) is an oxygenase involved in the synthesis of a β-lactam antibiotic from E. carotovora (McGowan, S., et al., *Mol Microbiol.*, 1996, 22:415-426; Khaleeli N, T. C., and Busby RW, *Biochemistry*, 2000, 39:8666-8673). XylR (TrEMBL: O31397) and BH0900 (TrEMBL: Q9KEF2) are enhancer binding proteins involved in the regulation of pentose utilisation (Rodionov, D., et al., *FEMS Microbiol Lett.*, 2001, 205:305-314) in bacillaceae and clostridiaceae. The comparison of FGE and DUF323 led to the establishment of a homology threshold differentiating the FGE family from distant DUF323-containing homologs with different functions. The latter include a serine/threonine kinase and XylR, a transcription enhancer as well as FGE, a *FGly* generating enzyme and CarC, an oxygenase. As discussed in elsewhere herein, FGE might also exert its cysteine

modifying function as an oxygenase, suggesting that FGE and non-FGE members of the DUF323 seed may share an oxygenase function.

Homologs of FGE

The presence of closely related homologs of human FGE in the DUF323 seed directed us to search for homologs of human FGE in NCBI's NR database (Wheeler et al., *Nucleic Acids Res.*, 2002, 20:13-16). The threshold of the search was chosen in such a way that all 6 homologs present in the DUF323 seed and other closely related homologs were obtained without finding the other seed members. This search led to the identification of three FGE orthologs in eukaryotes, 12 orthologs in prokaryotes and two paralogs in man and mouse (Table 3).

...

Note that the mouse sequence GI 22122361 is predicted in GenBank to encode a protein of 284 aa, although the cDNA sequence NM 145937 encodes for a protein of 372 residues. This misprediction is based on the omission of the first exon of the murine FGE gene. All sequences found in the NR database are from higher eukaryotes or prokaryotes. FGE-homologs were not detected in archaebacteriae or plants. Searches with even lowered thresholds in the fully sequenced genomes of *C. elegans* and *S. cerevisiae* and the related ORF databases did not reveal any homologs. A search in the eukaryotic sequences of the NT and EST nucleotide databases led to the identification of 8 additional FGE orthologous ESTs with 3'-terminal cDNA sequence fragments showing a high degree of conservation on the protein level which are not listed in the NR database. These sequences do not encompass the full coding part of the mRNAs and are all from higher eukaryotes (Table 4).

...

Multiple alignment and construction of a phylogenetic tree (using ClustalW) of the coding sequences from the NR database allowed the definition of four subgroups of homologs: eukaryotic orthologs (human, mouse, mosquito and fruitfly FGE, eukaryotic paralogs (human and mouse FGE paralog), prokaryotic orthologs closely related to FGE (Streptomyces and Corynebacterium and other prokaryotic orthologs (Caulobacter, Pseudomonas, Mycobacterium, Prochlorococcus, Mesorhizobium, Sinorhizobium, Novosphingobium, Ralstonia, Burkholderia, and Microscilla) . The eukaryotic orthologs show an overall identity to human FGE of 87% (mouse), 48% (fruitfly) and 47% (anopheles). While FGE orthologs are found in prokaryotes and higher eukaryotes, they are missing in the completely sequenced genomes of lower eukaryotes phylogenetically situated between S. cerevisiae and D. melanogaster. In addition, FGE homologs are absent in the fully sequenced genomes of E. coli and the pufferfish.

As discussed elsewhere herein, the FGE paralogs found in human and mouse may have a minor *FGly*-generating activity and contribute to the residual activities of sulfatases found in MSD patients.

Subdomains of FGE

The members of the FGE gene family have three highly conserved parts/domains (as described elsewhere herein). In addition to the two non-conserved sequences separating the former, they have non-conserved extensions at the N- and C- terminus. The three conserved parts are considered to represent subdomains of the DUF323 domain because they are spaced by non-conserved parts of varying length. The length of the part spacing subdomains 1 and 2 varies between 22 and 29 residues and that spacing subdomains 2 and 3 between 7 to 38 amino acids. The N- and C-terminal non-conserved parts show an even stronger variation in length (N-terminal: 0-90 AA, Cterminal: 0-28 AA). The sequence for the FGE gene from Ralstonia metallidurans is probably incomplete as it lacks the first subdomain.

To verify the plausibility of defining subdomains of DUF323, we performed a secondary structure prediction of the human FGE protein using Psipred. The hydrophobic ER-signal (residues 1-33) is predicted to contain helix-structures confirming the signal prediction of the von-Heijne algorithm. The N-terminal non-conserved region (aa 34-89) and the spacing region between subdomains 2 and 3 (aa 308-327) contain coiled sections. The region spacing subdomains 1 and 2 contains a coil. The α-helix at aa 65/66 has a low predicition confidence and is probably a prediction artefact. The subdomain boundaries are situated within coils and do not interrupt α-helices or β-strands. The first subdomain is made up of several β-strands and an α-helix, the second subdomain contains two β-strands and four α-helices. The third subdomain has a α-helix region flanked by a sheet a the beginning and the end of the subdomain. In summary, the secondary structure is in agreement with the proposed subdomain structure as the subdomain boundaries are situated within coils and the subdomains contain structural elements α-helices and β-strands).

It should be noted that none of the subdomains exists as an isolated module in sequences listed in databases. Within each of the four subgroups of the FGE family, the subdomains are highly conserved, with the third subdomain showing the highest homology (Table 5). This subdomain shows also the strongest homology across the subgroups.

...

The first subdomain of the FGE-family shows the weakest homology across the subgroups. In the eukaryotic orthologs it carries the N-glycosylation site: at residue Asn 141 in human, at Asn 139 in the mouse and Asn 120 in the fruit fly. In anopheles, no asparagine is found at the residue 130 homologous to D. melanogaster Asn 120. However, a change of two nucleotides would create an N-glycosylation site Asn 130 in anopheles. Therefore, the sequence encompassing residue 130 needs to be resequenced. The second subdomain is rich in tryptophans with 12 Trp in 129 residues of human FGE. Ten of these tryptophans are conserved in the FGE family.

High conservation of subdomain 3: subdomain 3 between eukaryotic orthologs are 100% similar and 90% identical. The importance of the third subdomain for the function of the protein is underlined by the observation that this subdomain is a hot spot for disease causing mutations in MSD patients. Seven of nine mutations identified in six MSD patients described in Example 1 are located in sequences that encode the 40 residues of subdomain 3. The residues contain four cysteines, three of which are conserved among the pro- and eukaryotic orthologs. The two eukaryotic paralogs show the lowest homology to the other members of the FGE-family, e.g. they lack two of the three conserved cysteines of subdomain 3. Features conserved between subdomain 3 sequences of orthologs and paralogs are the initial RVXXGG(A)S motif (SEQ ID NO:79), a heptamer containing three arginines (residues 19-25 of the subdomain consensus sequence) and the terminal GFR motif. A comparison with the DUF323 domain of the 15 seed sequences that are no close homologs of FGE shows marked sequence differences: the 15 seed sequences have a less conserved first and second subdomain, although the overall subdomain structure is also visible. Subdomain 3, which is strongly conserved in the FGE family, is shorter and has a significantly weaker homology to the eukaryotic subdomain 3 (similarity of about 20%) as compared to the prokaryotic FGE family members (similarity of about 60%). Thus they lack all of the conserved cysteine residues of subdomain 3. The only conserved features are the initial RVXXGG(A)S motif (SEQ ID NO:79) and the terminal GFR motif.

Genomic organisation of the human and murine FGE gene

The human FGE gene is located on chromosome 3p26. It encompasses 105 kb and 9 exons for the translated sequence. The murine FGE gene has a length of 80 Kb and is located on chromosome 6E2. The 9 exons of the murine FGE gene have nearly the same size as the human exons (Figure 3). Major differences between the human and the

mouse gene are the lower conservation of the 3'-UTR in exon 9 and the length of exon 9, which is 461 bp longer in the murine gene. Segment 6E2 of mouse chromosome 6 is highly syntenic to the human chromosome segment 3p26. Towards the telomere, both the human and the murine FGE loci are flanked by the genes coding for LMCD1, KIAA0212, ITPR1, AXCAM, and IL5RA. In the centromeric direction, both FGE loci are flanked by the loci of CAV3 and OXTR.

Genomic organisation of the prokaryotic FGE genes

In prokaryotes the sulfatases are classified either as cysteine- or serine-type sulfatases depending on the residue that is converted to *FGly* in their active center (Miech, C., et al., *J Biol Chem.*, 1998, 273:4835-4837; Dierks, T., et al., *J Biol Chem.*, 1998, 273:25560-25564). In Klebsiella pneumoniae, E. coli and Yersinia pestis, the serine-type sulfatases are part of an operon with AtsB, which encodes a cytosolic protein containing iron-sulfur cluster motifs and is critical for the generation of *FGly* from serine residues (Marquordt, C., et al., *J Biol Chem.*, 2003, 278:2212-2218; Szameit, C., et al., *J Biol Chem.*, 1999, 274:15375-15381).

It was therefore of interest to examine whether prokaryotic FGE genes are localized in proximity to cysteine-type sulfatases that are the substrates of FGE. Among the prokaryotic FGE genes shown in Table 3, seven have fully sequenced genomes allowing a neighbourhood analysis of the FGE loci. Indeed, in four of the 7 genomes (C. efficiens: PID 25028125, P. putida: PID 26990068, C. crescentus: PID 16125425 and M. tuberculosis: PID 15607852) a cysteine-type sulfatase is found in direct vicinity of FGE compatible with a cotranscription of FGE and the sulfatase. In two of them (C. efficiens and P. putida), FGE and the sulfatase have even overlapping ORFs, strongly pointing to their coexpression. Furthermore, the genomic neighbourhood of FGE and sulfatase genes in four prokaryotes provides additional evidence for the assumption that the bacterial FGEs are functional orthologs.

The remaining three organisms do contain cysteine-type sulfatases (S. coelicolor: PID 24413927, M. loti: PID 13476324, S. meliloti: PIDs 16262963, 16263377, 15964702), however, the genes neighbouring FGE in these organisms neither contain a canonical sulfatase signature (Dierks, T., et al., *J Biol Chem.*, 1998, 273:25560-25564) nor a domain that would indicate their function. In these organims the expression of FGE and cysteine-type sulfatases is therefore likely to be regulated *in trans*.

Conclusions

The identification of human FGE whose deficiency causes the autosomal-recessively transmitted lysosomal storage disease Multiple Sulfatase Deficiency, allows the definition of a new gene family which comprises FGE orthologs from prokaryotes and eukaryotes as well as an FGE paralog in mouse and man. FGE is not found in the fully sequenced genomes of E. coli, S. cerevisiae, C. elegans and Fugu rubripes. In addition, there is a phylogenetic gap between prokaryotes and higher eukaryotes with FGE lacking in any species phylogenetically situated between prokaryotes and D. melanogaster. However, some of these lower eukaryotes, e.g. C. elegans, have cysteine-type sulfatase genes. This points to the existence of a second *FGly* generating system acting on cysteine-type sulfatases. This assumption is supported by the observation that E. coli, which lacks FGE, can generate *FGly* in cysteine-type sulfatases (Dierks, T., et al., *J Biol Chem.*, 1998, 273:25560-25564).

Thus, the specification makes it clear that Applicants had identified and characterized essential features of Formylglycine Generating Enzyme that are present in orthologs of SEQ ID NO:2. Based on this detailed information setting out the exact criteria by which Applicants identified numerous orthologs (and paralogs) of human Formylglycine Generating Enzyme, a

skilled practitioner would clearly recognize that Applicants were in possession of the claimed Formylglycine Generating Enzyme of SEQ ID NO:2 and orthologs thereof. Applicants respectfully request that this rejection of claims 86-90 be withdrawn.

Applicants have added new claims 101-115. These claims recite a Formylglycine Generating Enzyme with at least 95% identity SEQ ID NO:2 or a Formylglycine Generating Enzyme encoded by a nucleic acid that hybridizes under stringent conditions (6X SSC at 65°C) to the complement of a nucleic acid encoding SEQ ID NO:2, and further recite that the polypeptide possesses a function of the protein of SEQ ID NO:2 (formation of L-C_α-formylglycine on a sulfatase). Applicants submit that these claims are analogous to Examples 9 and 14 of the Synopsis of Application of Written Description Guidelines ("Guidelines")[1] and thus satisfy the written description requirement.

Enablement

Applicants thank the Office for stating at page 8 that the specification enables a sulfatase-producing cell transformed with an expression vector encoding a sulfatase polypeptide and encoding the FGE polypeptide of SEQ ID NO:2, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, or 78, wherein the FGE polypeptide modifies a catalytic cysteine to a formylglycine of the sulfatase.

However, at pages 8-14 of the Office Action, the Office alleges that claims 86-90 are not enabled, as the full scope of the Formylglycine Generating Enzyme recited in the claims is not enabled. Applicants respectfully disagree, and address the Wands factors discussed by the Office in turn.

The breadth of the claims. The Office states at page 10, "The sequence of the FGE has been interpreted as being unlimited."

In the interest of expediting prosecution, Applicants have amended claim 86 to recite a Formylglycine Generating Enzyme of SEQ ID NO:2 or an ortholog thereof. The specification provides ample guidance regarding the sequence of SEQ ID NO:2, an analysis of its subdomains and conserved sequences, a description of how to identify orthologs and paralogs from

eukaryotes and prokaryotes, and provides numerous examples of orthologs. As described at length and in great detail in Examples 1 and 2 of the specification (pages 66-88), Applicants identified a new gene family and set forth how the members of the family were identified. Example 2 sets forth the exact criteria identified by Applicants that is used to determine whether or not an amino acid sequence encodes a member of the family (e.g., an ortholog). The criteria can be used, and in fact were used by Applicants, to identify orthologs of the Formylglycine Generating Enzyme of SEQ ID NO:2. Indeed, the specification sets forth the exact criteria to be used and provides working examples of applying the criteria. The criteria were effective to identify 18 family members in eukaryotes and prokaryotes and also to identify ortholog EST fragments in eukaryotes (see, e.g., Tables 4 and 5 of the specification).

Further, the specification teaches at length how to prepare and/or isolate homologs of the disclosed Formylglycine Generating Enzyme nucleic acid and amino acid sequences. See, for example, page 21, line 25- page 25, line 34; page 27, line 17- page 28, line 12; page 32, line 11- page 35, line 7. Thus, a skilled practitioner could apply the methodologies taught in the specification without undue experimentation to practice the claimed subject matter.

The Office also states on page 10, "the method of 'increased expression' of a sulfatase and FGE is unlimited ..." Indeed, Applicants submit that numerous methods can be used to increase expression of Formylglycine Generating Enzyme in a sulfatase-producing cell. Such methods are known in the art and described in the specification. For example, the specification describes vectors (e.g., targeting and/or expression vectors), such as plasmids and retroviral vectors, that can be used to increase expression of Formylglycine Generating Enzyme in a cell, the use of specific gene expression sequences such as regulatory sequences and promoters, and various types of cells that can be used (see, e.g., page 28, line 13- page 32, line 10; and page 48, line 6- page 51, line 12). The specification also teaches various methods for introducing Formylglycine Generating Enzyme-encoding sequences into cells (see, e.g., page 51, line 7- page 54, line 22). The specification teaches how to measure Formylglycine Generating Enzyme expression (see, e.g., page 39, lines 3-19). Further, the specification provides working examples of these methods (see, e.g., Examples 1-5 at pages 66-102 of the specification). Thus, a skilled practitioner could increase expression of a Formylglycine Generating Enzyme in a cell without undue experimentation.

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[1] The guidelines are available at http://www.uspto.gov/web/menu/written.pdf.

The state of the prior art, the level of one of ordinary skill, and the level of predictability in the art. The Office at pages 10-12 alleges that the level of unpredictability in altering a polypeptide sequence is high.[2] The Office cites several references, including Dierks et al. (*Cell* 113:435-444 (2003)), to support its position that the level of unpredictability in altering a polypeptide sequence is high. The Office at page 12 states that Dierks et al. "teaches that even single amino acid mutations in the *SUMF1* gene, which encodes human FGE, result in multiple sulfatase deficiency, which is characterized by a catalytically inactive FGE polypeptide."

Indeed, like the disclosure of the present specification (see, e.g., Table 1), Dierks et al. identifies mutations in human Formylglycine Generating Enzyme that are present in patients with multiple sulfatase deficiency. However, in contrast to the Office's position, the identification of these key residues supports the enablement of the claimed subject matter. By identifying the mutations present in human Formylglycine Generating Enzyme that are present in patients with multiple sulfatase deficiency, Applicants have identified key residues in the enzyme. As indicated in the specification (page 79, lines 14-16), the mutations in these patients occur in highly conserved residues in high conserved subdomains:

> All mutations have severe effects on the FGE protein by replacing highly conserved residues in subdomain 3 (three mutations) or subdomain 2 (one mutation) or C-terminal truncations of various lengths (four mutations) or large inframe deletions (two mutations).

As further stated on page 86, lines 18-22:

> High conservation of subdomain 3: subdomain 3 between eukaryotic orthologs are 100% similar and 90% identical. The importance of the third subdomain for the function of the protein is underlined by the observation that this subdomain is a hot spot for disease causing mutations in MSD patients. Seven of nine mutations identified in six MSD patients described in Example 1 are located in sequences that encode the 40 residues of subdomain 3.

Thus, Applicants' identification of mutations in human Formylglycine Generating Enzyme that are present in patients with multiple sulfatase deficiency is a further example of Applicants' thorough characterization of the enzyme and teaches the skilled practitioner which residues are important for enzyme function.

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[2] In taking its position, the Office cites to Branden et al., alleging that this reference has previously been cited. Applicants point out that the Office Action dated September 19, 2007 was the first Office Action on the merits for the present application and that a Branden et al. reference has not previously been cited. Applicants request that the Office cite this reference on a PTO-892 form and provide a copy of the reference if the Office relies on it further.

The amount of direction provided by the inventor and the existence of working examples. The Office alleges at pages 12-13 that "the specification fails to provide any additional *specific* guidance regarding those amino acids of an FGE polypeptide that can be altered with an expectation of maintaining the ability to modify a sulfatase catalytic cysteine to a formylglycine."

Applicants disagree. The specification teaches the structure of the Formylglycine Generating Enzyme protein, including the presence of three highly conserved subdomains, specific conserved motifs and residues within the subdomains, and the secondary structure of the subdomains and spacer regions (see, e.g., page 74, line 1- page 75, line 6; page 85, line 10- page 86, line 28). For example,

> The residues contain four cysteines, three of which are conserved among the pro- and eukaryotic orthologs. The two eukaryotic paralogs show the lowest homology to the other members of the FGE-family, e.g. they lack two of the three conserved cysteines of subdomain 3. Features conserved between subdomain 3 sequences of orthologs and paralogs are the initial RVXXGG(A)S motif (SEQ ID NO:79), a heptamer containing three arginines (residues 19-25 of the subdomain consensus sequence) and the terminal GFR motif. (page 86, lines 23-28)

Further, as stated above, the specification provides guidance and working examples regarding how to prepare and test homologs of the disclosed Formylglycine Generating Enzyme nucleic acid and amino acid sequences, and how to introduce mutations (e.g., conservative mutations) into the sequences. See page 21, line 25- page 25, line 34; page 27, line 17- page 28, line 12; page 32, line 11- page 35, line 7. Also as stated above, the specification identifies mutations present in human Formylglycine Generating Enzyme in patients with multiple sulfatase deficiency, thereby teaching which residues must be conserved in SEQ ID NO:2 (human Formylglycine Generating Enzyme). Thus, contrary to the Office's allegations, the specification is replete with guidance regarding amino acids, subdomains, and structures that are important for Formylglycine Generating Enzyme function.

The Office further alleges at page 13, "the specification fails to provide any specific guidance for modifying a cell to achieve overexpression of a sulfatase and an FGE."

Applicants disagree. Methods for overexpressing a protein in a cell (e.g., a eukaryotic or prokaryotic cell) are well known in the art. Further, as discussed above, the specification gives ample guidance regarding how to overexpress a protein, such as Formylglycine Generating

Enzyme, in a cell. For example, vectors (e.g., targeting and/or expression vectors), such as plasmids and retroviral vectors, can be used to increase expression of Formylglycine Generating Enzyme in a cell, specific gene expression sequences such as regulatory sequences and promoters, and various types of cells can be used (see, e.g., page 28, line 13- page 32, line 10; and page 48, line 6- page 51, line 12). The specification also teaches various methods for introducing Formylglycine Generating Enzyme-encoding sequences into cells , such as colloidal dispersion systems, liposomes (with or without targeting molecules), microparticles, implants, polymer matrices, compaction agents, transfection, viral (e.g., retroviral) infection, time-released and time-delayed delivery systems (see, e.g., page 51, line 7- page 54, line 22; page 59, line 3- page 60, line 28). The specification teaches how to measure Formylglycine Generating Enzyme expression (see, e.g., page 39, lines 3-19). Further, the specification provides working examples of these methods (see, e.g., Examples 1-5 at pages 66-102 of the specification). Indeed, experiments performed in Example 1 of the specification used retroviral gene transfer to transduce cells with Formylglycine Generating Enzyme, experiments performed in Example 3 of the specification transfected cells with expression vectors to increase Formylglycine Generating Enzyme expression, and experiments performed in Example 4 of the specification used microcell mediated chromosome transfer to increase Formylglycine Generating Enzyme expression in cells. Based on the guidance provided in the specification and the level of skill in the art, a skilled practitioner could certainly increase Formylglycine Generating Enzyme expression in a cell without undue experimentation.

The quantity of experimentation needed to make or use the invention based on the content of the disclosure. The Office at page 13 alleges:

> It was not routine in the art at the time of the invention to screen for any FGE as broadly as encompassed by the claims for those that have the ability to maintain activity of modifying a sulfatase catalytic cysteine to a formylglycine and to overexpress this FGE along with any sulfatase in a host cell by any method of overexpression as broadly encompassed by the claims.

As discussed above, the specification teaches at great length and in great detail how to isolate and/or prepare the Formylglycine Generating Enzyme of SEQ ID NO:2 and orthologs thereof, as recited in the amended claims. Also as discussed above, the specification provides

guidance and working examples of methods that can be used to overexpress a Formylglycine Generating Enzyme in a cell.

Applicants submit that based on the teachings of the specification and the level of skill in the art at the time the invention was made, the claimed subject matter could be practiced by a skilled practitioner without undue experimentation. For at least the reasons provided herein, Applicants respectfully request that the enablement rejection of claims 86-90 be withdrawn.

35 U.S.C. § 102

Szameit et al. (J. Biol. Chem. 274:15375-15381 (1999); "Szameit"). The Office alleges that claims 86-90 are anticipated by, or obvious in light of, Szameit, as evidenced by Fang et al. (J. Biol. Chem. 279:14570-14578 (2004); "Fang").

Applicants respectfully disagree. Claim 86 has been amended to recite a Formylglycine Generating Enzyme that is an activated form of an endogenous Formylglycine Generating Enzyme of SEQ ID NO:2 or an ortholog thereof or an exogenous Formylglycine Generating Enzyme of SEQ ID NO:2 or an ortholog thereof. Further, as indicated in the specification, the Formylglycine Generating Enzyme of SEQ ID NO:2 and its orthologs modify a cysteine residue in sulfatases:

> As mentioned elsewhere herein, Schmidt et al. first discovered that sulfatases undergo a post-translational modification of a highly conserved cysteine, that is found at the active site of most sulfatases, to Cα-formylglycine. They also showed that this modification was defective in MSD (Schmidt, B., et al., *Cell*, 1995, 82:271-278). Our mutational and functional data provide strong evidence that FGE (SUMF1) is responsible for this modification. (page 99, lines 6-11; emphasis added)

In contrast, the sulfatase-modifying enzyme described in Szameit modifies serine resides. As stated in the specification:

> In Klebsiella pneumoniae, *E. coli* and *Yersinia pestis*, the serine-type sulfatases are part of an operon with AtsB, which encodes a cytosolic protein containing iron-sulfur cluster motifs and is critical for the generation of *FGly* from serine residues (Marquordt, C., et al., *J Biol Chem.*, 2003, 278:2212-2218; Szameit, C., et al., *J Biol Chem.*, 1999, 274:15375-15381). (page 87, lines 24-27; emphasis added)

Indeed, this is confirmed by Fang and the Office acknowledges the same on page 16 of the Office Action. Thus, the disclosure of Szameit pertains to an enzyme that modifies serine residues, rather than cysteine residues. Therefore, Szameit does not teach or suggest the same Formylglycine Generating Enzymes as currently claimed. For at least this reason, Szameit does

not anticipate or render obvious the claimed subject matter. Applicants respectfully request that this rejection of claims 86-90 be withdrawn.

Rommerskirch et al. (Proc. Natl. Acad. Sci USA 89:2561-2565 (1992); "Rommerskirch"). The Office alleges that claims 86-90 are anticipated by, or obvious in light of, Rommerskirch, as evidenced by Dierks et al. (*Cell* 113:435-444 (2003); "Dierks").

Applicants respectfully disagree. Amended claim 86 recites a sulfatase-producing cell which expresses a Formylglycine Generating Enzyme that is **an activated form of an endogenous** Formylglycine Generating Enzyme of SEQ ID NO:2 or an ortholog thereof or **an exogenous** Formylglycine Generating Enzyme of SEQ ID NO:2 or an ortholog thereof, wherein expression of the Formylglycine Generating Enzyme is increased as compared to expression in the same cell type **without** the activated form of the Formylglycine Generating Enzyme.

In contrast, in Rommerskirch, a comparison was made between cells that *lack* an activity (multiple sulfatase deficiency (MSD) fibroblasts) and cells that do *not* lack the activity (normal fibroblasts or fibroblasts with a single sulfatase deficiency). There was no teaching or suggestion whatsoever regarding an endogenous Formylglycine Generating Enzyme being activated or that an exogenous Formylglycine Generating Enzyme being expressed in a cell. Rather, Rommerskirch merely concluded that the mutation present in MSD fibroblasts "decreases the capacity of a co- or post-translational process" (Rommerskirch Abstract). From this disclosure, a skilled practitioner would not have had any rational reason to attempt to activate an endogenous Formylglycine Generating Enzyme or to express an exogenous Formylglycine Generating Enzyme in a cell. The Office's allegation that Dierks "provides evidence that MSD fibroblasts are defective in FGE activity" does not remedy this deficiency.

Further, the claim recites that the ratio of active sulfatase to total sulfatase produced by the cell is increased by at least 5% as compared to the ratio in the same cell type without the activated form of the endogenous Formylglycine Generating Enzyme of SEQ ID NO:2 or the ortholog thereof or the exogenous Formylglycine Generating Enzyme of SEQ ID NO:2 or the ortholog thereof. Because Rommerskirch fails to offer any teaching or suggestion pertaining to activating an endogenous Formylglycine Generating Enzyme or expressing an exogenous Formylglycine Generating Enzyme in a cell, it also fails to anticipate or render obvious a cell in which the ratio of active sulfatase to total sulfatase produced by the cell is increased by at least

5% as compared to the ratio in the same cell type without the activated form of the endogenous Formylglycine Generating Enzyme or the exogenous Formylglycine Generating Enzyme. The evidence from Dierks fails to remedy this deficiency.

For at least these reasons, Rommerskirch does not anticipate or render obvious the claimed subject matter. Applicants respectfully request that this rejection of claims 86-90 be withdrawn.

CONCLUSION

For at least the reasons stated above, Applicants respectfully submit that all pending claims are in condition for allowance, which action is expeditiously requested. Applicants do not concede any positions of the Examiner that are not expressly addressed above, nor do Applicants concede that there are not other good reasons for patentability of the presented claims or other claims.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. Please charge any deficiency to Deposit Account No. 50/2762.

Respectfully submitted,
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